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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Philippe SANSONETTI et al.	Group Art Unit: 1645
Application No.: 08/466,698	Examiner: Albert M. NAVARRO
Filed: June 6, 1995	
For: METHOD FOR PRODUCING TRANSFORMED *SHIGELLA* (As Amended)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF JEAN-MICHEL ALONSO, M.D., PH.D., UNDER 37 C.F.R. § 1.132

I, Jean-Michel Alonso, M.D., Ph.D., do hereby make the following declaration:

1. My curriculum vitae, and a list of publications that I have authored or coauthored, is attached hereto as Exhibit A.

2. As shown on my curriculum vitae, I have extensive experience in field of molecular genetics of pathogenic bacteria. I have been employed at Institut Pasteur in Paris, France, since 1978. Since 1998 I have been head of the *Neisseria* Unit at Institut Pasteur and of the French National Reference Center for the Meningococci.

3. During the period of 1983-1988 I was Head of the Bordetella laboratory in the Bacterial Ecology Unit, Department of Bacteriology and Mycology, at Institut Pasteur. During that period, my work was focused on the use of bacterial molecular genetics to understand bacterial virulence factors, and the immunogenicity of bacterial antigens.

4. On information and belief, attached hereto as Exhibit B is a copy of U.S. patent application Serial No. 08/466,698 ("the '698 application").

5. I have read and understood the contents of the attached copy of the '698 application.

6. On information and belief, the '698 application was filed on June 6, 1995.

7. On information and belief, the '698 application claims the benefit of priority of European Patent Application Serial No. 88 401 842.5, which was filed on July 15, 1988.

8. On information and belief, attached hereto as Exhibit C is a copy of "Proposed Claims for U.S. Patent Application Serial No. 08/466,698" ("the proposed claims").

9. On information and belief, attached hereto as Exhibit D is a copy of a journal article published in the August 15, 1986, issue of Cell, authored by Makino et al., and entitled "A Genetic Determinant Required for Continuous Reinfection of Adjacent Cells on Large Plasmid in *S. flexneri* 2a" ("Makino").

10. I have read and understood the contents of the attached copy of Makino.

11. I am submitting this Declaration to explain the phenotypic characterization of *virG* mutant *S. flexneri* described in Makino. (Because *virG* and *icsA* are different names for the same gene, and because the '698 application and the proposed claims refer to the gene as *icsA*, I will refer to the gene as *icsA* throughout this Declaration, including in reference to Makino.) In particular, I will explain the significance of the phenotypic characterization provided by Makino to the suitability of a modified *Shigella* comprising an inactivated *icsA* for use in making a vaccine against a wild strain of *Shigella*.

12. Makino discloses a modified *Shigella*, comprising an *icsA* gene inactivated by insertion of a transposon (an "inactivated *icsA* gene"). (Exhibit D at page 554.) According to Makino, *Shigella* comprising an inactivated *icsA* gene can invade host cells and multiply within host cells, "but do not proceed further." (Exhibit D at page 554, left column.) Specifically, Makino describes *Shigella* comprising an inactivated *icsA* gene as being "extinguished before they can spread and infect adjacent cells." (Exhibit D at page 551, right column.) Thus, according to Makino, *Shigella* comprising an inactivated *icsA* gene retain the ability to invade host cells, but have lost the ability to spread from infected to uninfected host cells.

13. Makino also notes that, "[a]lthough multiplication occurs, the [*Shigella* comprising an inactivated *icsA* gene] lack active movement, show a tendency to localize within the cytoplasm, are gradually converted to a spherical morphology, and are finally extinguished from the epithelia." (Exhibit D at page

554, left column.) Thus, according to Makino, *Shigella* comprising an inactivated *icsA* gene have also lost the ability to spread within infected host cells.

14. The concept of a "live attenuated stain" was widely known as of July 15, 1988. A live attenuated strain is a strain modified by mutation of one or more genes to eliminate its pathogenicity, but not the ability of the strain to elicit a protective immune response. Such strains were known as of July 15, 1988. For example, the "International Dictionary of Medicine and Biology", published in 1986, defined an "attenuated vaccine" at page 3083 as "A live bacterial or viral vaccine, carrying mutations that eliminate its pathogenicity but not its ability to elicit a protective immune response." (A copy of the relevant pages of International Dictionary of Medicine and Biology (1986) is attached hereto as Exhibit E.)

15. As of July 15, 1988, it was known that making a live attenuated *Shigella* strain would require modifying a wild *Shigella* strain by mutating one or more genes required for pathogenicity of the wild strain, to create a modified strain that will invade and multiply in a host, but, unlike the corresponding wild strain, will not cause a disease pathology. It was appreciated that, while attenuation of the live attenuated strain is critical to render the strain non pathogenic, it is imperative that the strain retain some ability to invade, multiply, and spread within an inoculated host, so that the strain elicits a significant enough immune response to confer immunity to the wild strain to the host.

16. For this reason, based on the teachings of Makino, I would not expect that a modified *Shigella* strain comprising an inactivated *icsA* gene would

be useful as a live attenuated strain for making a vaccine against the wild *Shigella* strain.

17. I am aware that Makino states that modified *Shigella* strains comprising an inactivated *icsA* gene "may be a plausible candidate for a live vaccine against bacillary dysentery." (Exhibit D at page 554, left col.) I disagree with this statement. In fact, this assertion is clearly contrary to the description of the modified *Shigella* strain comprising an inactivated *icsA* gene provided by Makino. According to Makino, the modified strain is unable to survive in cells or tissues and does not spread within or between cells. For this reason, the strain would not be expected to elicit a robust immune response and would not be effective for making a vaccine.

18. In summary, based on the disclosure in Makino, and based on what was known about the molecular genetics of pathogenic bacteria as of July 15, 1988, I would not have been motivated to include an inactivated *icsA* gene in a modified *Shigella* strain for use in making a vaccine. To the contrary, I would have assumed that inclusion of an inactivated *icsA* gene in such a strain would have rendered it ineffective in making a vaccine against a wild strain of *Shigella.*

19. I have read the Declaration of Stewart Thomas Cole, Ph.D., Under 37 C.F.R. § 1.132, a copy of which is attached hereto as Exhibit F.

20. The language in the proposed claims regarding modified *Shigella* comprising an inactivated *icsA* gene differs significantly from the description in Makino. I agree with the opinion of Dr. Cole, that the recitation of "[a] method for modifying a wild strain of an enteroinvasive *Shigella* to produce a modified strain

of *Shigella* that can not spread substantially within infected cells of a host and can not spread substantially from infected to uninfected cells of the host, for use in making a vaccine against the wild strain of *Shigella*. . . ," in the proposed claims, must mean that the ability of the strain to spread within infected host cells, and from infected to uninfected host cells, is substantially reduced; but that the ability of the strain to spread within infected host cells, and from infected to uninfected host cells, is clearly not abolished. If it were, the modified strain would not be useful to make a vaccine against the wild strain of *Shigella*, which, according to the language of the proposed claims is the purpose of the modified strain.

21. In view of this language, and in view of the description of modified *Shigella* comprising an inactivated *icsA* gene provided by the '698 application, I would conclude that a modified *Shigella* comprising an inactivated *icsA* gene would be useful to make a vaccine against a wild strain of *Shigella*.

22. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 9/01/04

By: Jean Michel Alonso
Jean-Michel Alonso, M.D., Ph.D.